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TRAIL, LIGHT, and the Expanding Tumor Necrosis Factor Superfamily in Human Placentas

RYAN M. GILL[†], HAKHYUN KA[‡] and JOAN S. HUNT[†‡*]
[†]*Department of Pathology and Laboratory Medicine,* [‡]*Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS, 66160-7400*

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The tumor necrosis factor (TNF) superfamily comprises a group of powerful cytokines that in part regulate the magnitude of an immune response. Messages encoding virtually all members of this family are detected in human placentas and two prototypical superfamily members, TNFα and FasL, have been implicated in both normal and abnormal reproductive phenotypes. Here, further evidence for the reproductive importance of the TNF superfamily is examined by presenting recent findings that TNF Related Apoptosis Inducing Ligand (TRAIL) and "homologous to Lymphotoxin, exhibits Inducible expression, competes with herpes simplex virus Glycoprotein D for HVEM, a receptor expressed by T lymphocytes" (LIGHT), proteins are expressed and are biologically active in human placentas. The potential reproductive role(s) for members of this superfamily and unresolved issues of expression, regulation and function are discussed.

Key Words: Placenta, TNF, TRAIL, LIGHT

Introduction

The immune system has evolved to recognize and protect the vertebrate from foreign organisms and cancer. An elegant system educates immature immune cells to distinguish self from non-self. The placenta, often termed a semiallograft, represents an interesting obstacle to this system since it is derived from both maternal and paternal alleles. Normally, foreign (allogeneic) alleles if translated into proteins are perceived as non-self by the host immune system. If this were the case in the human placenta, maternal recognition of paternally derived molecules would predictably lead to classical graft rejection and pregnancy termination.

Placentation is highly variable among species with the human placenta representing one of the most invasive types. Invasion by placental cells results in direct contact with maternal blood and tissues. The fact that there is no evidence of graft rejection during normal pregnancy implies that the placenta enjoys privileged protection from the maternal immune system (Billingham & Medawar 1953). The mother and fetus appear cooperatively to down-regulate the functions of immune cells that are important for graft rejection while allowing innate host immune defenses to stay intact (reviewed in Wegmann & Guilbert 1992, Wegmann et al. 1993). This control is flexible and dynamic, facilitating defense from pathogens as the need arises. Because of its proximity to tissue-bound and circulating maternal immune cells, the placental trophoblast cell clearly has the responsibility for immune privilege. This is true in early gestation (figure 1a) and at termination (figure 1b).

The discovery and characterization of tumor necrosis factor-α (TNFα), and other members of the TNF superfamily in trophoblast cells have provided important insights into immunologic regulation at the maternal-fetal interface (Yang et al. 1993, reviewed in Hunt et al. 1996). Evidence acquired in other systems strongly suggests that the placenta can utilize these powerful cytokines to facilitate placental growth and development (reviewed in Hunt et al. 1996).

In this article we review the expression of TNF Related Apoptosis Inducing Ligand (TRAIL) and a molecule termed "homologous to Lymphotoxin, exhibits Inducible expression, competes with herpes simplex virus Glycoprotein D for HVEM, a receptor

*Corresponding Author: *E-mail:* JHUNT@kumc.edu; *Tel:* (913) 588-7270; *FAX:* (913) 588-7180

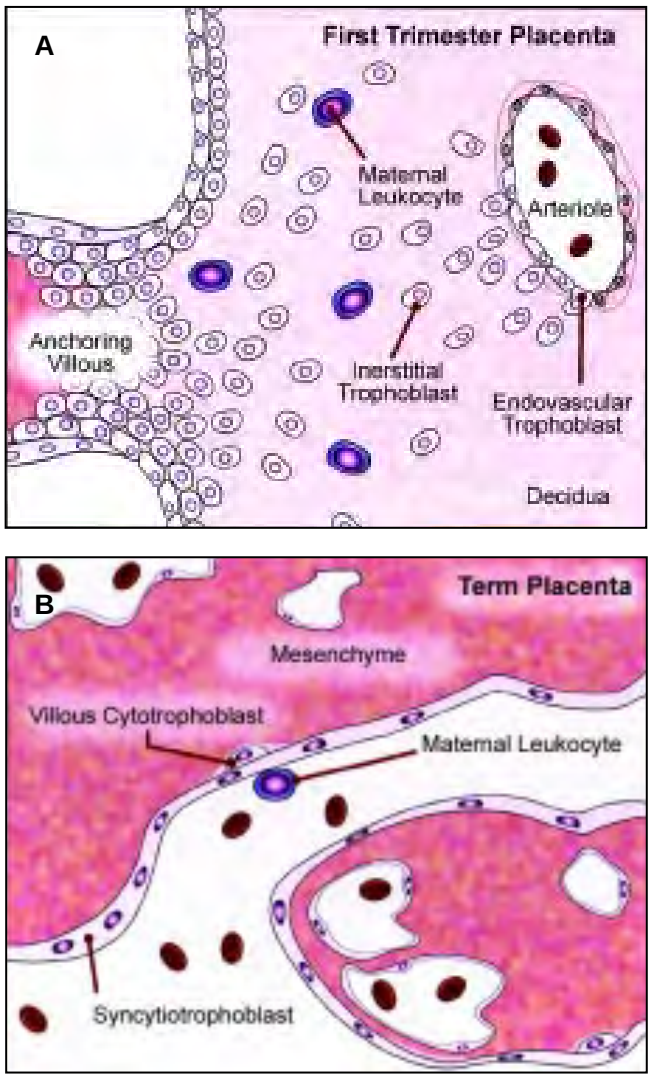

Figure 1. A schematic drawing illustrating first trimester (A) and term (B) placental anatomy.

expressed by T lymphocytes" (LIGHT) and their receptors in human placentas, and comment on evidence collected to date for immune modulation by these mediators as well as implications for roles in placental homeostasis. Finally, we present ideas and speculations on the delicate balance between placental privilege, growth/development, and response to circulating and uterine pathogens, based largely on the expanding data collected in other contexts regarding the influence of the TNF gene superfamily.

Background

Placenta

To appreciate the paradox of placental immune privilege it is useful to review the cell types that comprise the human placenta. Trophoblast cells are the major cells of placenta and separate from the inner cell mass at the blastocyst stage prior to implantation. There are three general types of trophoblast cells. Villous cytotrophoblast cells are precursors to the multinucleate syncytiotrophoblast layer and have stem cell-like properties. The syncytiotrophoblast layer secretes a variety of hormones that maintain pregnancy and ensure a hospitable environment for the fetus. Intermediate/extravillous cytotrophoblast cells invade the endometrium and replace the endothelial cells lining the maternal spiral arteries (figure 1A). Near the end of the first trimester maternal blood enters the intervillous space and contacts the syncytiotrophoblast layer (Jauniaux et al. 2000) (figure 1B). Mesenchymal cells, including fibroblast cells and placental macrophages (Hofbauer cells), along with fetal vasculature, populate the interior mesenchyme of placental villi.

Innate and Adaptive Immunity

Concepts of innate and adaptive immunity (reviewed by Janeway & Medzhitov 2002) are important to any discussion of immune privilege. The innate, or natural, branch of the immune system provides pathogen resistance through anatomic/physiologic, phagocytic cell, and inflammatory barriers.

The adaptive, or specific, branch of the immune system provides diverse and specific recognition of non-self proteins, or antigens. This branch employs several very specialized cells: T lymphocytes, B lymphocytes and antigen presenting cells (APCs). The two types of lymphocytes have distinct cell surface receptors highly specific for individual antigens. T cell receptors (TcR) and membrane bound antibodies (B cell receptors) are extremely diverse and can respond to virtually every antigen.

Communication between cells that orchestrate inflammatory responses is largely accomplished by a group of secreted glycoproteins called cytokines. Cytokines generally act over short distances and are often described as autocrine or paracrine mediators. Cytokines classically exhibit pleiotropy (cell dependent biological effects), redundancy (multiple cytokines trigger the same cellular response) and synergy/antagonism (action of one cytokine influences the response to another). Cytokine structure generally falls into one of the following families/superfamilies: TNF, chemokine, interferon, or hematopoietin, which includes the interleukins (IL). Interestingly, many cytokines have been identified in non-hematopoietic cells; prominent

examples in the human placenta include IL-6 (Kameda et al. 1990) and TNFa (Chen et al. 1991).

T Cells

T cells are generally divided into two major subsets, the CD4+ helper T cell (Th) and the CD8+ cytotoxic/suppressor T cell (CTL). Th cells can be activated to produce a number of cytokines. Th cells that are driven to produce cytokines that favor antigen presenting cell (APC) and CTL activation [IL-2, lymphotoxin-a (LTα), interferon-γ (IFNγ)] are termed Th1 cells while the Th cells that produce cytokines that favor B cell activation and innate immunity (IL-3, IL-4, IL-10) are designated Th2 cells. One mechanism of cytotoxicity used by CTLs is the expression of TNF superfamily members that initiate lethal caspase activation in target cells (Ashkenazi & Dixit 1999). This is a loose classification as there are cells that span the two categories or bridge relationships with other cell types such as Th0 and natural killer-T (NKT) cells.

CD8+ T cells recognize class I human leukocyte antigens (HLAs), which are present on virtually every cell. CD4+ T cells associate with class II HLAs, which are normally present only on APCs (macrophages, B cells, dendritic cells).

B Cells

B cells mature into plasma cells that produce clones of antibodies that can neutralize toxins, activate complement, initiate chemotaxis and enhance phagocytosis (opsonization).

Immune System and the Placenta

Innate Immunity and Human Pregnancy

Initial implantation in mammals with hemochorial placentation is associated with inflammation and local chemokine production, which recruits phagocytic cells [mostly polymorphonuclear leukocytes (neutrophils and eosinophils)] to this site (Murdoch & Finn 2000). Inflammatory cytokines such as TNFα, IL-1, and IFNγ are elevated during the first few days of murine implantation (reviewed in Hunt & Johnson 1997). During the progesterone driven transition of human endometrium to decidua, in accord with declining specific immunity, the T and B lymphocyte numbers decrease but the innate immune cell numbers increase [macrophage and natural killer (NK)-like cells] (Bulmer et al. 1995). The macrophages remain throughout gestation but the NK-like cells (CD16-/CD56+) disappear during the second trimester (King et al. 1998).

It is important to note that two components of mucosal immunity remain robust in the decidua, the mucosal T cells [a subset of T cells that have different TcR than non-mucosal T cells (γ/d vs α/β, respectively)] and the NKT lymphocytes, which share properties of both NK cells and T cells (Dang & Heyborne 2001, Mincheva-Nilsson et al. 1997)

Adaptive Immunity and Pregnancy

Cytokine Networks

Murine pregnancy reportedly favors Th2 predominance with down-regulation of CTL responses and up-regulation of antibody responses (Wegmann et al. 1993). Since the Th1 suppressive cytokine, IL-10, as well as the immunosuppressive molecules, TGFβ and prostaglandin E2, are elevated in the blood of pregnant women and rodents, there may be mechanisms in place to enforce this Th2 balance (reviewed in Hunt & Johnson 1997). It is important to note that Th1 cytokines are still present in placenta and it is likely that these proteins are important for placental development and function (Hunt et al. 1996, Gill et al. 2002).

HLAs

Trophoblast cells are known to protect themselves from CTL recognition by regulating their expression of HLAs (Hunt & Orr 1992, Le Bouteiller & Mallet 1997, Ober 1998). Not surprisingly, CTLs directed against paternal HLAs are difficult to detect in pregnant women (Sargent & Redman 1985). Class II HLAs are not expressed by trophoblast cells; and nonclassical class I HLAs with low polymorphisms (such as HLA-E, -F and -G) predominate in certain subpopulations of trophoblast cells (reviewed in Hunt & Johnson 1997).

Complement

Although maternal antibodies against paternal antigens are detected, complement mediated lysis of placental cells is unknown. Membrane cofactor protein (MCP or CD46) and decay accelerating factor (DAF) are implicated in defending human placenta from complement-mediated lysis (Hsi et al. 1991, Holmes et al. 1990). Dramatic evidence for the importance of these complement regulatory proteins comes from a recent report that mice lacking the *Crry* gene lose their pregnancies due to activation of maternal complement fixing antibodies (Xu et al. 2000).

Immune Privilege

Medawar's assessment of the immunologic contradiction of fetal survival in a nonidentical host (the mother), proposed three explanations: (i) maternal tolerance, (ii) maternal-fetal anatomic barriers and (iii) a lack of fetal immunogenicity (Billingham & Medawar 1953). Tolerogenic mechanisms now generally believed to be involved in pregnancy include HLA-G-mediated inhibition of immune cells, production of lymphocyte-inhibiting indoleamine 2,3-dioxygenase, regulation of the Th1/Th2 balance, suppression by macrophages, inhibition by the hormones of pregnancy, FasL/Fas-mediated apoptosis, lowered complement activity, and antigen camouflage (reviewed in Hunt & Tung 2001).

Although the placenta represents a semiallograft where foreign (paternal) antigens are unable to elicit classical graft rejection, this immune privilege is not a simple systemic down-regulation of maternal cell-mediated immunity; the mother remains immuno-competent throughout gestation (reviewed in Mellor & Munn 2000). Therefore, the mechanisms that allow placental privilege must also permit, directly or indirectly, a rapid response to maternal/placental pathogens. None of the current theories solely account for this balance between privilege and defense and many aspects of each theory are likely important. The pleiotropic TNF superfamily holds promise for bridging these diverse requirements.

The TNF Superfamily

Although TNF superfamily members are established immunomodulators, they are emerging as powerful mediators of gene expression unrelated to the immune system in diverse areas such as organ and tissue development (reviewed in Locksley et al. 2001). One of the first tissues in which TNFα was suggested to play a nonimmunologic role was the placenta (Hunt et al. 1990, Yelavarthi et al. 1991, Chen et al. 1991). Messages encoding nearly all TNF superfamily members are detected in early and late gestation placentas (Phillips et al. 2001). Among the ligands, at least eight are reported to cause apoptosis in a variety of cells (Phillips et al. 2001). Conversely, a majority of superfamily members can recruit intracellular adapters that permit nuclear factor-κB (NF-κB) activation and translocation into the nucleus (reviewed in Wallach et al. 1999), which protects against apoptosis. These observations may account for the superfamily member's capacity to both activate and suppress gene expression, depending on cell type and other conditions, and might allow for the highly modifiable and dynamic responses unfolding at the maternal-fetal interface over the course of normal pregnancy. Phillips et al. (2001) suggests that this superfamily is important in the regulation of placental growth, immune privilege and protection from carcinogenesis.

The best-studied members of the TNF superfamily are TNFα and FasL. For a detailed description on the reproductive role of these mediators, we refer the reader to several excellent reviews (Terranova et al. 1995, Hunt et al. 1996, Argiles et al. 1997, Hunt & Rasmussen 1998, Hunt et al. 1999).

TNFα and the Placenta

Normal Placentas

Although a large bolus of TNFα initiates hemorrhage and pregnancy termination, TNFα is normally detected in the human placenta, uterus, oviduct and embryo when no pathology is evident (reviewed in Hunt et al. 1996). TNFα message and protein have been detected in the first trimester and term syncytiotrophoblast layer, decidual cells, endovascular trophoblast cells, term villous mesenchymal cells, and in the chorion at termination (Chen et al. 1991). Message has also been detected in first trimester villous and proliferating cytotrophoblast cells (Yang et al. 1993), purified term cytotrophoblast cells and in the choriocarcinoma-derived cell lines Jar and JEG-3 (Phillips et al. 2001, Yang et al. 1993). Evidence that TNFα may be used in autocrine trophoblast cell signaling comes from antisense and antagonist TNF receptor (TNFR) 1 antibody experiments on Jar cells (Yang et al. 1993). Evidence also exists that term placental macrophages may be important producers of TNFα (Chen et al. 1991), especially during labor (Steinborn et al. 1998).

TNFα has been demonstrated to be important for cell and tissue homeostasis and is thought to play a positive role in reproduction (reviewed in Hunt et al. 1996). TNFα promotes apoptosis in human villous cytotrophoblast cells especially in conjunction with IFNγ treatment and may be important in villous remodeling (Yui et al. 1994) or syncytial differentiation/fusion (Huppertz et al. 1998, 1999). TNFα is also thought to play a role in apoptosis of

extravillous trophoblast cells (Reister et al. 2001). TNFα can increase the production of matrix metalloproteinase 9 (MMP-9) in early gestation trophoblast cells, possibly facilitating their invasion into decidua (Meisser et al. 1999). Interestingly, MMP-9 is not affected by TNFα at term but urokinase type plasminogen activator, a protease indicative of an invasive phenotype, is increased significantly (Monzon-Bordonaba et al. 2002). Monzon-Bordonaba et al. (2002) also find that TNFα treatment reduces β-hCG production in cultured term cytotrophoblast cells, an observation previously reported in first trimester placental explants (Ohashi et al.1992).

Although TNFα-/- mice do not show an overt reproductive phenotype they are significantly more susceptible to *Listeria monocytogenes*, an intracellular placental pathogen (Pasparakis et al. 1996). TNFα-/-LTα-/- mice, in addition to disorganized spleen (Eugster et al. 1996), show abnormal labyrinthine architecture, but again no overt reproductive phenotype (Rasmussen et al. 1997). Evidence for a TNF superfamily role in normal human placental biology comes from a report that TNFα message and protein, in the syncytiotrophoblast layer, are reduced in some instances of suboptimal fertility (Lea et al. 1997).

Pathological Placentas

Elevated Th1 cytokines, such as TNFα, in the blood, amniotic fluid or decidua have been correlated with human (Casey et al. 1989, Romero et al. 1989, Casey et al. 1990, Hill et al. 1995, Raghupathy 1997, Piccinni et al. 1998) and murine miscarriages (Haddad et al. 1997). Although the overall contribution of TNF superfamily members to female fertility remains to be determined, one report observed increased TNFα in cervical mucus from women with idiopathic infertility (Naz et al. 1995). Other placental pathologies associated with TNFα include chorioamnionitis (Dollner et al. 2002), preterm premature rupture of membranes (PPROM) (Roberts et al. 1999) and preeclampsia (Benyo et al. 2001).

FasL and the Placenta

FasL is a powerful inducer of apoptosis that functions to down-regulate lymphocyte proliferation and limit immune responses (reviewed in Nagata & Golstein 1995, Jacobson et al. 1997, Barinaga 1998, Van Parjis et al. 1998). The syncytiotrophoblast layer expresses FasL (Runic et al. 1996). This observation has prompted the suggestion that FasL plays a role in maintaining the immune privileged status of placenta by killing activated maternal lymphocytes (Runic et al. 1996). Runic et al. (1998) also demonstrated that human fetal membranes contain Fas and exhibit signs of apoptosis, indicating an additional potential role in fetal membrane remodeling. However, given that trophoblast cells also express Fas (Xerri et al. 1997, Runic et al. 1998, Payne et al. 1999), and since murine models do not as yet support a role for FasL in placental immune privilege (Hunt et al. 1997), its function in the placenta is still obscure. One recent report illustrates the power of cytokine networks by demonstrating that Th2 cytokines increase resistance of trophoblast cells to Fas-mediated apoptosis (Aschkenazi et al. 2002).

Summary

Infertility represents a major health problem; approximately one in six couples is infertile as reported by the ISLAT Working Group (1998). Placental pathology contributes to many cases of early pregnancy loss and TNFα is thought to be important in many of these pathologies.

Although a definitive role in placental biology has not yet emerged for either TNFα or FasL, considerable experimental evidence supports the idea that TNFα is important for placental homeostasis, villous remodeling, cytotrophoblast cell differentiation and defense from placental pathogens. Although TNFα -/- mice do not exhibit an overt phenotype, clinical associations do exist in the human, suggesting that redundant mechanisms compensate for TNFα's function in the placenta. There is significant structural and functional overlap in the immunoregulatory functions of the TNF superfamily and, as with TNFα, messages encoding most TNF superfamily and TNFR superfamily members are present in human placentas. Thus the observations made to date justify a thorough investigation of the localization, function, and modulation of the TNF/TNFR superfamily signaling pathways in human placenta.

TRAIL

TRAIL was originally discovered by its homology to the DNA sequence of the conserved region of the TNF superfamily. This superfamily ligand has been shown to induce apoptosis in various tumor cells,

but not in normal cells (Wiley et al. 1995, Pitti et al. 1996), which has led to its development as an anti-cancer therapeutic. TRAIL can also kill activated lymphocytes (Martinez-Lorenzo et al. 1998) and antigen-presenting macrophages (Kaplan et al. 2000), implying a role in immune privilege. This section will discuss the features and possible roles of TRAIL in placental immune privilege.

Cellular Immunology of TRAIL

Among the members of the TNF superfamily TRAIL shares the highest sequence homology with FasL (Wiley et al. 1995, Pitti et al. 1996). Although TRAIL may be a membrane-bound ligand, a soluble form of TRAIL also exists due to cleavage by cysteine proteases (Mariani & Krammer 1998). Biologically active TRAIL exists as a homotrimer and requires a zinc ion for stability and bioactivity (Hymowitz et al. 2000). Like FasL and some other members of the TNF superfamily, TRAIL is capable of inducing apoptosis (Degli-Esposti 1999).

TRAIL mRNA is detectable in various human tissues, predominantly in spleen, lung, thymus and prostate (Wiley et al. 1995). TRAIL expression is upregulated by various factors, including TNFα (Siegmund et al. 2001), IFNα (Sato et al. 2001), IFNβ (Sato et al. 2001), IFNγ (Phillips et al. 1999), IL-2 (Kayagaki et al. 1999), IL-10 (Sohn et al. 2001), IL-15 (Kayagaki et al. 1999) and progesterone (Popovici et al. 2000). It has been shown that various immune cells express TRAIL. T and B cells express TRAIL on their cell surface in an activation-dependent manner, and treatment with a protein kinase C activator (phorbol 12-myristate 13-acetate), calcium ionophore (ionomycin), phytohemagglutinin or anti-CD3/CD28 antibodies can upregulate expression of TRAIL in T cells (Mariani & Krammer 1998, Baetu et al. 2001). IL-2 and IL-15 activated murine NK cells also express TRAIL (Kayagaki et al. 1999). Recent results show that the TRAIL gene, which is composed of five exons and four introns, has five single nucleotide polymorphisms in the coding region, including one in exon 1, one in exon 5 and three in the 3' UTR. These results suggest possible allelic influences on the TRAIL gene (Gray et al. 2001).

Cellular Immunology of TRAIL Receptors

Five receptors are known to bind TRAIL (figure 2). These are death receptor-4 (DR4; also called TRAIL-R1), DR5 (also called TRAIL-R2, TRICK2 or KILLER), decoy receptor-1 (DcR1; also called TRAIL-R3, TRID or LIT), DcR2 (also called TRAIL-R4 or TRUNDD) and osteoprotegerin (OPG) (Ashkenazi & Dixit 1998, Degli-Esposti 1999). DR4 and DR5 are both highly homologous to Fas and TNFR-1, and are involved in transducing apoptosis-inducing signals through an intracellular death domain (Wiley et al. 1995). DR4 and DR5 signaling can also activate NF-κB, a transcription factor, which is known to protect cells from undergoing apoptosis (Sheridan et al. 1997). DcR1 is bound to the cell surface via a glycosyl-phosphatidylinositol (GPI) anchor and lacks a transmembrane and intracellular domain (Degli-Esposti et al. 1997a, 1997b). DcR2 contains an intracellular domain, but is unable to transduce apoptotic signals due to its incomplete cytoplasmic death domain. Therefore, it has been suggested that DcR1 and DcR2 protect normal cells from TRAIL-induced apoptosis by competing with DR4 and DR5 for TRAIL binding (Baker et al. 1997, Degli-Esposti et al. 1997a, 1997b, Marsters et al. 1997a, Pan et al. 1997, Sheridan et al. 1997).

TRAIL Intracellular Signaling

The best-defined function of TRAIL is apoptosis induction via DR4 and DR5, which contain the death domain (Ashkenazi & Dixit 1998, Degli-Esposti 1999). The apoptosis signaling pathway triggered by DR4 and DR5 involves the activation of adaptor proteins, such as TNF-receptor-associated factors (TRAFs), Fas-associated death domain protein (FADD), TNFR-associated death domain protein (TRADD), receptor-interacting protein (RIP) and caspase-8 (Chaudhary et al. 1997, Schneider et al. 1997, Kischkel et al. 2000, Lin et al. 2000, Sprick et al. 2000). FADD and caspase-8 are essential for DR4- and DR5-induced apoptosis (Schneider et al. 1997, Sprick et al. 2000), and TRADD, RIP and TRAF-2 are required to activate NF-κB and c-Jun NH_2-terminal kinase (JNK) (Lee et al. 1997, Degli-Esposti 1999, Lin et al. 2000). DR4 and DR5 activate NF-κB in a cell type-dependent manner (Chaudhary et al. 1997, Sheridan et al. 1997). Additionally, p38 mitogen-activated protein kinase (MAPK) can be activated by TRAIL suggesting that this cytokine may signal through yet another pathway (Chou et al. 2001). Understanding TRAIL signaling is complicated by the presence of multiple receptors, multiple intracellular signaling pathways, and activation of multiple inhibitory molecules (figure 2).

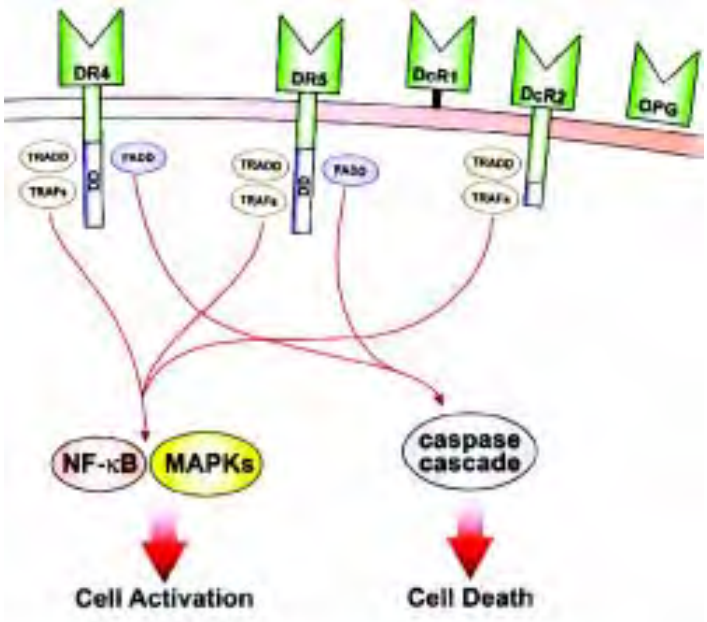

Figure 2. A schematic illustration of TRAIL receptors and intracellular signaling pathways. *DR*, death receptor; *DcR*, decoy receptor; *OPG*, osteoprotegerin; *FADD*, Fas-associated death domain protein; *TRADD*, tumor necrosis factor receptor-associated death domain protein; *TRAFs*, tumor necrosis factor receptor-associated factors; *NF-κB*, nuclear factor-κB; *MAPKs*, mitogen-activated protein kinases.

TRAIL and Apoptosis

Although TRAIL was first reported to induce apoptosis in various tumor cell lines (Wiley et al. 1995), it has been suggested that TRAIL mediates various biological activities, such as macrophage homeostasis (Kaplan et al. 2000), regulation of erythropoiesis (Zamai et al. 2000), NK cell cytotoxicity (Kayagaki et al. 1999, Sato et al. 2001, Takeda et al. 2001) and inhibition of autoimmune inflammation (Song et al. 2000). A recent study using TRAIL -/- mice showed that TRAIL plays a role in suppressing tumor initiation and metastasis (Cretney et al. 2002).

TRAIL and its Receptors in the Placenta

Expression of TRAIL and its receptors in placenta has thus far only been reported in humans (Phillips et al. 1999). TRAIL transcripts and proteins are detectable in first trimester placentas, term human placentas and trophoblastic cell lines (Jar and JEG-3). Immunoreactive TRAIL is strongly expressed in the syncytiotrophoblast layer, placental macrophages, amniotic membranes and maternal decidual cells. Receptors for TRAIL are expressed in first trimester and term placentas. Villous cytotrophoblast cells isolated from term placentas contain DR4, DR5, DcR1 and DcR2, but not OPG mRNAs, suggesting that TRAIL may modulate cytotrophoblast cell function (Phillips et al. 2001).

Regulation

Studies on trophoblast cell lines (Jar and JEG-3) showed that steady-state levels of TRAIL mRNA are regulated by IFNγ (Phillips et al. 1999). A recent report demonstrates that progesterone and/or cyclic AMP (cAMP) stimulate TRAIL expression in endometrial stromal cells, suggesting that the decidualization process upregulates TRAIL expression (Popovici et al. 2000). However, the effect of progesterone and cAMP on TRAIL expression in isolated trophoblast cells, cultured *in vitro*, has not been tested. Other types of regulation, which may influence TRAIL mRNA stability, are suggested by our finding of three polymorphisms in the 3'UTR of the TRAIL gene (Gray et al. 2001).

Function

The expression pattern of TRAIL and its receptors in placentas show clearly that TRAIL is positioned on trophoblast cells to kill leukocytes circulating in maternal blood (Phillips et al. 1999). TRAIL may therefore have a critical role in defending the syncytiotrophoblast layer against attack by maternal blood leukocytes, thus conferring placental immune privilege. As described above, villous cytotrophoblast cells, which are positioned directly below the syncytiotrophoblast layer, express all membrane-bound TRAIL receptors. These data suggest that in addition to contributing to placental immune privilege, TRAIL may influence cytotrophoblast cell functions (Phillips et al. 2001). Preliminary studies analyzing cDNA cytokine arrays revealed that TRAIL modulates expression of various genes, including growth factors, in human term placental villous cytotrophoblast cells (Ka et al. 2002). These results indicate that TRAIL may have a major impact on cytotrophoblast cell proliferation and/or differentiation in human placenta.

LIGHT

Although much is known of the involvement of LIGHT in the human immune system, nothing is yet known about its function in pregnancy.

Cellular Immunology of LIGHT

LIGHT, as with other members in this superfamily, forms a homotrimer and has both cytosolic and membrane forms (Harrop et al. 1998, Zhai et al. 1998, Granger et al. 2001). The membrane form can be cleaved by matrix metalloproteinases to act as a soluble protein (Morel et al. 2000, Tamada et al. 2000a, Granger et al. 2001).

It has been reported that LIGHT is required for primary allogeneic T cell responses (Tamada et al. 2000a) and is important in the induction of cell-mediated immunity (Tamada et al. 2000a, b, Morel et al. 2001, Wang et al. 2001). LIGHT, as with most members of the TNF superfamily, has the ability to trigger apoptosis in some tumor cells in culture and *in vivo* (Chen et al. 2000, Tamada et al. 2000b).

Observations relevant to pregnancy include a report that organs of transgenic mice, which constitutively express LIGHT under control of the CD2 promoter, show significant pathology including abnormal lymphoid architecture, intestinal inflammation, and atrophied uterine horns (Shaikh et al. 2001). These mice exhibit decreased fertility (Shaikh et al. 2001). Studies in LIGHT -/- mice verify that LIGHT is critical for T cell costimulation and suggest that LIGHT acts cooperatively with LTb in lymphoid organogenesis (Scheu et al. 2002, Tamada et al. 2002). Gross infertility has not been observed in the LIGHT -/- mouse model (Scheu et al. 2002, Tamada et al. 2002, Ye et al. 2002).

Cellular Immunology of LIGHT Receptors

The increasingly varied roles that LIGHT could play emphasize that its ultimate function will depend on receptor expression and the local cytokine environment. LIGHT is known to bind three receptors, herpes virus entry mediator (HVEM), lymphotoxin-β-receptor (LTβR), and decoy receptor-3 (DcR3)/M68/TR6. These receptors show the functional overlap that is characteristic of the TNFR superfamily, as they bind other members of the TNF superfamily (figure 3).

HVEM is present on resting T cells and is important in LIGHT-mediated costimulation (Montgomery et al. 1996, Mauri et al.1998, Zhai et al. 1998, Tamada et al. 2000a). LTβR is not found on T and B cells but is found on monocytes and some tumor cells in which it transduces apoptotic signals (Mauri et al. 1998, Wu et al. 1999). LTβR is important for peripheral lymphoid organogenesis in mice (DeTogni et al. 1994, Rennert et al. 1996, Koni et al. 1997, Futterer et al. 1998).

DcR3 is a soluble protein found in a variety of human tissues that can compete with HVEM for LIGHT binding (Pitti et al. 1998, Yu et al. 1999, Zhang et al. 2001). DcR3 not only blocks LIGHT mediated apoptosis and T cell activation but can shift the Th1 cytokine balance associated with LIGHT towards the Th2 type (Yu et al. 1999, Bai et al. 2000, Roth et al. 2001, Zhang et al. 2001). Hsu et al. (2002) report that DcR3 promotes a Th2 response through modulation of dendritic cell maturation and differentiation.

LIGHT Intracellular Signaling

TRAF-2 and TRAF-5 are intracellular proteins that associate with activated HVEM (Marsters et al. 1997b, Rooney et al. 2000). Similarly, TRAF-5 and TRAF-3 associate with activated LTβR (Van Arsdale et al. 1997, Rooney et al. 2000). Although many intracellular signaling molecules are likely involved (Wallach et al. 1999), it is clear that TRAF-2 and

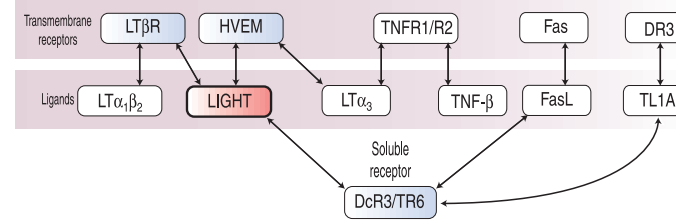

Figure 3. A schematic illustration of LIGHT and its related tumor necrosis factor (TNF) superfamily members. Arrows indicate receptor-ligand interactions. *LT*, lymphotoxin; *LTβR*, lymphotoxin-β-receptor; *TNFR*, tumor necrosis factor receptor; *DR*, death receptor; *DcR*, decoy receptor; *DcR3/TR6*, decoy receptor 3; *HVEM*, herpes virus entry mediator; *TL1A*, TNF like factor-1A; *LIGHT*, "homologous to Lymphotoxin, exhibits Inducible expression, competes with herpes simplex virus Glycoprotein D for HVEM, a receptor expressed by T-lymphocytes." Modified from Gill et al. (2002).

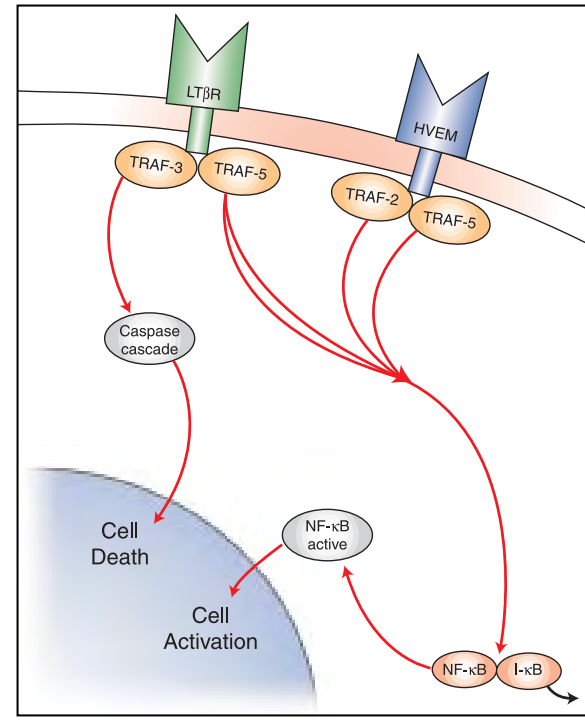

Figure 4. A schematic illustration of TNF receptor associated factor (TRAF) signaling in LIGHT-stimulated cells. LTβR, lymphotoxin-β-receptor; HVEM, herpes virus entry mediator; NF-κB, nuclear factor-κB.

TRAF-5 target the NF-κB system and that TRAF-3 targets the caspase cascade, which is one mechanism of apoptosis induction (figure 4).

LIGHT and Apoptosis

LIGHT, like other members of the TNF superfamily, has the noted ability to trigger apoptosis in some tumor cells in culture and *in vivo* (Tamada et al. 2000b). Early work showed that both LTβR and HVEM are required for apoptotic susceptibility in the PC-3 human prostate carcinoma cell line (Zhai et al. 1998). It was later shown that in some cell lines the presence of LTβR is sufficient for apoptotic signaling through TRAF-3 (Wu et al. 1999, Rooney et al. 2000).

LIGHT and its Receptors in the Placenta

We recently mapped the expression pattern of LIGHT and its receptors in term human placenta. We demonstrated that the syncytiotrophoblast layer expressed LIGHT, HVEM, LTβR and a lesser amount of DcR3 while the villous mesenchymal cells expressed only LIGHT, LTβR, and DcR3 proteins (Gill et al. 2002).

Despite evidence that LIGHT promotes inflammation, the placenta abundantly expresses this cytokine in areas that would permit its interaction with maternal immune cells. As such we have suggested that alternate functions for LIGHT may exist in the placenta. Proposed functions for placental LIGHT include defense from pathogens, placental homeostasis, and placental remodeling through apoptosis (Gill et al. 2002). It is perhaps even more likely that LIGHT will cause apoptosis in cytotrophoblast cells given their dual expression of HVEM and LTβR (Gill et al. 2002), which in other cell types favors apoptotic signaling (Zhai et al. 1998). This is especially interesting given recent experiments linking apoptosis with cytotrophoblast cell differentiation/syncytial fusion (Huppertz et al. 1998, 2001). It is likely that locally expressed soluble DcR3 modulates the function of placental LIGHT, as this protein favors the Th2 environment postulated to be important for normal pregnancy (Gill et al. 2002).

Perspectives

The presence of so many TNF superfamily members in the human placenta suggests that these powerful cytokines may act to promote a supportive immunologic environment for the placenta. Regulatory mechanisms, such as soluble and decoy receptors (DcR1, DcR2, DcR3) are in place to ensure that effector functions are tightly controlled. The similarities between LIGHT, TRAIL and TNFα also suggest a certain degree of redundancy and overlapping functions that may favor placental homeostasis.

Model Systems for Studying TNF Superfamily Function and Regulation in Placenta

In vivo models are undoubtedly the most powerful and knockouts of many TNF superfamily members are available. However, controversy exists as to whether significant correlations can be made between murine and human placentas (Rossant & Cross 2001, Moffett-King 2002).

Although several groups have generated TRAIL -/- and LIGHT -/- mice, none have reported gross infertility (Scheu et al. 2002, Tamada et al. 2002, Ye et al. 2002, Cretney et al. 2002). This is perhaps not surprising given that messages encoding virtually every ligand and receptor in the TNF superfamily are present in human placentas (Phillips et al. 2001) and many are also present in murine placentas (Crainie et al. 1999, Hunt et al. 1997). This expression pattern suggests that individual functions may overlap and compensate for a single knockout. Double knockouts have been generated, including TNFα-/-LTα-/- mice, which do show subtle placental phenotypes (Rasmussen et al. 1997).

Purified term human cytotrophoblast cells are a common *in vitro* model for studying the effects of TNF superfamily members on placental viability, development, and differentiation. Although the purification technique developed by Kliman et al. (1986) and later modified by Douglas and King (1989) is functional and widely used, the purified term cells do not proliferate and can only be cultured for several days. Nevertheless the Percoll gradient centrifugation step utilized by Kliman et al. remains an efficient means of removing syncytial fragments to obtain a very pure population of cytotrophoblast cells (1986), which can be further purified by immunomagnetic techniques (Phillips et al. 2001). Investigators interested in syncytial fragments often omit the Percoll gradient step (Huppertz et al. 1999). Other *in vitro* models exist that utilize an extracellular matrix and facilitate studying the invasive properties of isolated trophoblast cells (Fisher et al. 1989). Since TNFα is likely important in modulating the invasive phenotype of cytotrophoblast

cells (Meisser et al. 1999, Monzon-Bordonaba et al. 2002), this model may be appropriate for future TNF superfamily investigation.

The difficulty of working with human tissue is significant and many investigators have turned to immortalized trophoblastic cell lines. In general trophoblastic cell lines respond and function differently than their normal counterparts and are less useful for investigating normal physiology. For example, the Jar and JEG-3 choriocarcinoma derived cell lines respond mitogenically to TNFα (Yang et al. 1993), which is the opposite of the apoptosis reported in purified cytotrophoblast cells (Yui et al. 1994). The trophoblast-like cell line ED(27) is reportedly contaminated with HeLa cells and should no longer be used (Kniss et al. 2002). Extravillous trophoblast cells rapidly die in culture and a hybrid with the JEG-3 choriocarcinoma cell line has been utilized to investigate this trophoblast population (Frank et al. 2000).

Given the imperfections of current models, investigators can be expected to gravitate toward more realistic and controllable models of human pregnancy. Certain primates seem ideally suited for this role, notably the baboon, which appears to parallel closely the human in many aspects of reproduction (Langat et al. 2002).

Future Questions

Evidence accumulated to date suggests that the TNF and TNFR superfamily members are important for pregnancy and that deviation from normal expression levels can result in pathology. However, critical questions remain unanswered involving TNF superfamily function and regulation in human placenta. Specifically, much needs to be learned about TNF superfamily member function in hormone regulation, placentation and immune privilege at the maternal-fetal interface.

Much of the descriptive groundwork necessary to address these questions is in place. Current and future advances in proteomics and data analysis by bioinformatics should allow investigators to better understand the overall function of cytokine networks at the maternal fetal interface. Recent advances in the generation of small interfering RNAs (siRNA), which specifically degrade RNA or RNAs of interest, should permit more rigorous *in vitro* and possibly *in vivo* experimentation that will allow critical questions of differentiation and immune modulation to be better addressed. Nevertheless, resolving how expression of TNF superfamily members, in the context of all other placental immunomodulators, contributes to successful pregnancy remains a great and exciting challenge.

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